

UTILIZATION OF HYDROCARBONS BY FUNGI

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ABSTRACT - Utilization of benzene, kerosene and solar by fungi has been studied during 60 days period when incorporated in soil. The results of hydrocarbon-treated soils ■ two doses (5% and 40%) on glucose-agar (1%) with or without 1% hydrocarbon were nearly similar. Thirty species of fungi and one variety belonging to 14 genera were isolated from hydrocarbon free soil (11 genera, 24 species and 1 variety), benzene-(5 genera, 12 species and 1 variety), kerosene-(7 genera and 17 species) and solar-treated soils (8 genera, 19 species and 1 variety) on glucose-agar. *Amorphotheca resiniae*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Fusarium solani*, *Penicillium funiculosum*, *Rhizopus stolonifer* and *Trichoderma harzianum* were the most prevalent species of one or more hydrocarbons. The results obtained showed there are no hydrocarbon utilizing fungi characteristic of benzene, kerosene and solar.

RÉSUMÉ - L'utilisation par des champignons, de benzène, de kérosène ou de solar, incorporés dans le sol, a été étudiée sur une période de 60 jours. Les résultats obtenus sont sensiblement les mêmes, quand les sols sont traités par 5% ou 40% d'hydrocarbures et les isolements réalisés sur glucose-Agar (1%), complété ou non par 1% d'hydrocarbure. Au total, 30 espèces et 1 variété, appartenant à 14 genres ont été isolées du sol sans hydrocarbure (11 genres, 24 espèces, 1 variété), du sol traité par le benzène (5 genres, 12 espèces, 1 variété), du sol traité par le kérosène (7 genres, 17 espèces) et du sol traité par le solar (8 genres, 19 espèces, 1 variété). *Amorphotheca resiniae*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Fusarium solani*, *Penicillium funiculosum*, *Rhizopus stolonifer* et *Trichoderma harzianum* sont les espèces les plus couramment isolées sur un ou plusieurs des hydrocarbures étudiés. Aucune des espèces isolées n'est spécifique du benzène, du kérosène ou du solar.

INTRODUCTION

Microorganisms play an important role in natural removal of petroleum hydrocarbons from contaminated ecosystems. Fungi capable of metabolizing hydrocarbons are limited, with most reported to occur within the orders, Mucorales (Zygomycetes) and Moniliales (Hyphomycetes) (Nyns et al., 1968; Walker et al., 1976). Thus the ability of fungi and yeasts to utilize pure aliphatic hydrocarbon (n-alkanes) as sole carbon and energy sources is a well-documented phenomenon reviewed by Klug & Markovetz (1971). The fate of polycyclic aromatic hydrocarbons (PAH) has received attention recently since benzo (a) pyrene and similar compounds were shown to be toxic, carcinogenic and/or mutagenic. Fungi have been shown to oxidize PAH in a manner similar to that found in mammals (Cerniglia, 1981).

In Egypt, Hemida (1991) isolated several fungal species from oil-polluted soils collected from different gas stops. Recently, Bagy et al. (1992) reported on naphthalene-anthracene utilizing microorganisms (bacteria and fungi). The present investigation

was undertaken to study utilization of 3 hydrocarbons (benzene, kerosene and solar) by fungi.

MATERIALS AND METHODS

Hydrocarbon-treated soil

Clay soil collected from the University farm was used in this study. 500 g aliquot of air-dry sieved soil was placed in a polyethylene bag and thoroughly mixed with commercial benzene or kerosene or solar. The water content of the soil was adjusted to 28% W.H.C.. Each hydrocarbon was added to soil in two doses (5% and 40%). The treatments were set up in duplicates in addition to the control (untreated soil). They were then incubated at 28°C (+ 2°C) for 60 days. After 7, 30 and 60 days, soil samples were taken and assayed for their fungal counts.

Determination of hydrocarbon utilizing fungi

Each hydrocarbon-treated soil was analysed for its fungal population using the dilution plate method (Johnson & Curl, 1972) on glucose-Czapek's agar (10 g/L) with or without 1% of hydrocarbon. Rose bengal (66 ppm) was employed as a bacteriostatic agent. Incorporation of rose bengal in the soil dilution plate has also the advantage of restricting the size of spreading colonies of fungi and therefore, large numbers of fungal colonies appear on the plate. Five plates were used for each treatment and the control and they were incubated at 28°C (+ 2°C), for 7-15 days, during which the developing fungi were examined, identified and counted.

The following references were used for the identification of fungal genera and species: Raper & Thom (1949), Raper & Fennell (1965), Rifai (1969), Booth (1977) and Bomsch et al. (1980).

RESULTS AND DISCUSSION

Fungi recovered from hydrocarbon free soil

Twenty-four fungal species and one variety belonging to 11 genera were recovered from untreated soil (hydrocarbon free) on glucose-Czapek's agar (Tab. 1). The total count of fungi after 7 days was higher than the initial count and then the count decreased after incubation for 30 and 60 days. This means that, some fungi could increase their numbers in soil deprived from any additive organic substrate. It is possible that these fungi could utilize remains of available food-materials or could benefit from the secondary activities in soil. *Amorphotheca resinae*, *Aspergillus carneus*, *Mucor circinelloides*, *Myrothecium verrucaria*, *Penicillium steckii* and *Scopulariopsis candida* were completely absent in the control samples. However, these fungal species were isolated from hydrocarbon-treated soils.

Fungi recovered from benzene-treated soil

The mycological analysis of benzene-treated soil revealed the isolation of 12 fungal species and one variety belonging to 5 genera on glucose-agar (Tab. 1). These numbers are considerably lower than those obtained from untreated soil (24 species and 1 variety, 11 genera). Moreover, the total count of fungi was consistently lowered than the control one at both doses used after all the experimental periods. The reduction in the fungal count could be attributed to the toxic effect of benzene on soil

| Treatment | Untreated soil | | | | | | Benzene-treated soil | | | | | | Kerosene-treated soil | | | | | | Solar-treated soil | | | | | |
|--|----------------|------|------|------|------|------|----------------------|------|-----|-----|------|------|-----------------------|------|------|-----|------|------|--------------------|------|------|-----|---|--|
| | Dose | | | | | | Dose | | | | | | Dose | | | | | | Dose | | | | | |
| | 0% | | 5% | | 10% | | 5% | | 10% | | 5% | | 10% | | 5% | | 10% | | 5% | | 10% | | | |
| Period/day | 0 | 7 | 30 | 50 | 7 | 30 | 50 | 7 | 30 | 50 | 7 | 30 | 50 | 7 | 30 | 50 | 7 | 30 | 50 | 7 | 30 | 50 | | |
| Fungal species | | | | | | | | | | | | | | | | | | | | | | | | |
| Total count | 36.3 | 41.9 | 28.8 | 31.6 | 15.4 | 12.1 | 7.6 | 12.5 | 6.7 | 6.0 | 35.3 | 12.3 | 4.7 | 13.3 | 13.0 | 6.7 | 31.2 | 15.5 | 5.6 | 24.4 | 17.4 | 5.4 | | |
| <i>Ascomondium strictum</i> W. GAMS | - | 0.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Altamantia altamanta</i> (Fr.) KEGSSLER | - | 0.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Aspergillus nidulans</i> PARBURY | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Aspergillus stenocephalus</i> BERK. & CURT | - | - | - | 0.3 | - | - | - | - | - | - | - | - | - | 10.3 | 5.6 | - | 0.7 | 0.1 | - | 0.1 | - | - | - | |
| <i>Ascomondium</i> VAN TIEGHEM BLOCHWITZ | - | - | - | - | - | - | - | - | - | - | - | - | 0.3 | 0.3 | - | - | - | - | - | - | - | - | | |
| <i>A. niger</i> LINK | 3.7 | 1.3 | 7.0 | 4.8 | - | - | - | 0.3 | - | - | 2.0 | 0.1 | 0.1 | - | - | 0.2 | - | 0.1 | - | - | - | 1.0 | | |
| <i>A. niger</i> var. <i>colonyensis</i> PAPER & PENNELL | - | 1.3 | - | - | - | 0.1 | - | 0.3 | - | - | - | - | - | - | - | - | - | 0.1 | - | 1.4 | 0.3 | 1.0 | | |
| <i>A. fumigatus</i> FRESENIUS | 2.3 | - | - | 0.5 | - | 12.0 | - | - | - | - | - | - | 0.3 | 0.8 | 1.3 | - | 1.1 | - | - | - | - | - | | |
| <i>A. japonicus</i> SAITO | 0.3 | - | - | - | - | - | 4.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| <i>A. niger</i> VAN TIEGHEM | 19.7 | 25.3 | 11.7 | 16.5 | 7.0 | 0.1 | - | 5.3 | - | - | 24.5 | 6.3 | 2.9 | 8.3 | 2.9 | 2.3 | 22.1 | 8.5 | 2.0 | 13.3 | 5.1 | 0.7 | | |
| <i>A. terreus</i> THOM | 1.7 | 4.7 | 1.7 | 2.7 | 6.7 | - | 1.7 | 3.0 | - | - | 3.7 | 0.9 | 0.4 | 2.8 | - | - | 1.7 | 0.5 | 0.5 | 7.7 | 2.0 | 1.0 | | |
| <i>A. ustus</i> BAINIERI THOM & CHURCH | 0.7 | - | - | 1.8 | - | - | 1.0 | - | - | - | - | - | - | - | - | - | 0.7 | - | - | - | - | 0.1 | | |
| <i>Sterigmatella nidulans</i> (TEIGAMI) VUILLEMEN | - | 1.0 | 2.3 | 3.8 | - | - | - | 0.3 | - | - | 1.7 | 0.8 | 0.3 | 0.3 | - | - | 0.3 | 1.3 | 0.4 | - | 1.5 | 1.3 | | |
| <i>Basidium moniliforme</i> SHELTON | - | 0.7 | 0.2 | - | - | - | - | - | - | - | - | - | - | 0.2 | - | - | 1.3 | - | - | - | - | - | | |
| <i>Phoma</i> SCHLECHT | - | - | - | 0.3 | - | - | - | - | - | - | - | - | - | 0.1 | - | - | 1.3 | 0.1 | - | - | - | 0.3 | | |
| <i>Tricholoma</i> (MART.) SACC. | 0.7 | - | 0.2 | - | - | - | - | - | - | - | 0.7 | 0.1 | 0.1 | - | - | 0.1 | 0.3 | - | 0.1 | - | - | 0.1 | | |
| <i>Tricholoma cristallinum</i> VAN TIEGHEM | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| <i>A. niger</i> WEHNER | - | 0.3 | 0.3 | - | - | - | - | - | - | - | 0.3 | 0.1 | - | - | - | - | 1.7 | - | - | 0.3 | - | 0.1 | | |
| <i>A. niger</i> FRESENIUS | - | - | 0.3 | - | - | - | - | - | - | - | - | 0.1 | - | - | - | - | - | - | - | - | - | 0.1 | | |
| <i>Tricholoma verrucosum</i> (ALB.) SCHW. (DITM.) EX FR. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| <i>Tricholoma</i> (MART.) WEHNERI MALLOCCAN & CAIR D. J. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.1 | | |
| <i>Tricholoma chrysogenum</i> THOM | 1.7 | - | 2.7 | - | - | - | - | - | - | - | - | - | 3.3 | - | - | - | - | - | - | 0.7 | - | - | | |
| <i>P. funiculosum</i> THOM | 0.7 | - | 2.0 | 0.7 | 0.7 | - | - | 0.7 | - | - | 1.7 | - | - | 0.7 | - | - | - | - | - | - | - | - | | |
| <i>P. lelandicum</i> SCOP | - | 5.0 | - | - | 0.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.0 | 1.0 | 1.0 | | |
| <i>P. venosum</i> ZAIESKI | 1.0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| <i>P. omilium</i> CURRIE & THOM | - | - | 0.2 | - | 0.7 | - | - | 1.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| <i>P. atropicum</i> ZAIESKI | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| <i>Phaeopus atropicus</i> (FENBEN) LING | 1.0 | - | - | 0.2 | - | 0.1 | - | - | - | - | 0.3 | - | 0.1 | - | - | - | 0.3 | - | - | - | - | - | | |
| <i>Cooperomyces candida</i> (QUESLEN) VUILLEMEN | - | - | - | - | - | - | 0.7 | - | - | - | - | - | - | - | - | - | 0.1 | 0.1 | - | - | - | 0.1 | | |
| <i>Trichoderma harzianum</i> PIFAI | 0.7 | - | 0.2 | - | - | - | 0.3 | - | - | - | - | - | - | - | - | - | - | - | 0.3 | - | - | - | | |
| <i>Trichoderma reesei</i> (CORF) HUGHES | - | 0.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |

Table 1: Total counts (calculated per mg soil) of fungal species in soil treated with benzene or kerosene or solar on glucose-Czapek's agar at 28°C.

fungi. The above results were obtained on both glucose- and benzene- Czapek's agar (Tables 1, 2).

Six species and one variety of *Aspergillus* were isolated, these were *A. flavus* (at 40% after 7 days only), *A. flavus* var. *columnaris* (at 5% and 40% after 60 and 7 days, respectively), *A. fumigatus* (at 5% after 30 days and at 40% after 30 and 60 days), *A. japonicus* (at 5% after 60 days only), *A. niger* (at 5% after 7, 30 days and at 40% after 7 days), *A. terreus* (at 5% and 40% after 7, 60 days and 7 days, respectively) and *A. ustus* (at 5% after 60 days only). *Penicillium funiculosum*, *P. islandicum* and *P. oxalicum* were recovered at low and high doses after 7 days only. *Emericella nidulans*, *Scopulariopsis candida* and *Trichoderma harzianum* were isolated at 40% after 7 days. Fedorak & Westlake (1986) reported that isolates of *Paecilomyces*, *Verticillium*, *Beauveria* and *Penicillium* species were tested for ability to metabolize a variety of n-alkylbenzenes. Growth on dodecylbenzene yielded benzoic and phenylacetic acids ■ transient intermediates, and these acids supported growth of the isolates. Ratledge (1984) mentioned that microorganisms, i.e. bacteria, yeasts and moulds, can grow on a wide variety of hydrocarbons as sole sources of carbon and energy. They can partially oxidize an even greater range of such compounds. Also, he reported that, the list of compounds attacked is extensive and includes straight and branched chain alkanes, alkene, alicyclic, heterocyclic and aromatic hydrocarbons.

Fungi recovered from kerosene-treated soil

A total of 7 genera and 17 species were isolated from kerosene-treated soil on glucose-agar (Tab. 1). These numbers were higher than those obtained from benzene-treated soil. Also, the total count of fungi were regularly declined at both doses and with the lengthening of the experimental period, and the lowest count was estimated at 40% after 60 days.

Amorphotheca (= *Cladosporium*) *resinae* was isolated mostly from kerosene-treated soil at 40% after 30 and 60 days on both media (Tables 1, 2). In addition, it was isolated at 5% after 30 days on glucose-agar supplemented with 1% kerosene. The highest counts (10 and 12 colonies) of *A. resinae* were estimated at 40% after 30 days on glucose- and 1% kerosene-glucose-agar, respectively. Cabral (1980) reported that it was possible to verify the presence of *Cladosporium resinae* f. *avellaneum* as the principal contaminant in Jet fuel (Kerosene) from storage tanks, hose tips and aircraft integral fuel tanks. May & Neihof (1981) stated that, one of the most prevalent and troublesome contaminants found associated with fuels in contact with either freshwater or seawater is *C. resinae*. Smucker & Cooney (1981) studied the growth of *C. resinae* on glucose and then transferred to medium with glucose or with kerosene as the sole carbon source. Carson & Cooney (1988) observed that cells of *C. resinae* form greater numbers of microbodies when grown on n-alkanes than when grown on glucose. In this study, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Mucor hiemalis*, *M. racemosus*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Trichoderma harzianum* were decreased at both doses of kerosene but with variable degrees. However, *A. alutaceus*, *A. carneus*, *A. fumigatus*, *F. solani* and *P. funiculosum* were promoted at some treatments (Tab. 1). Bemmann & Voigt (1980) isolated thermophilic n-alkane assimilating hyphae fungi and strains of *Aspergillus fumigatus* and *Mucor lusitanicus* selected from physiological investigation. Hussein & Abdel-Gawad (1983) studied the protease and amylase activities of the marine fungus *A. flavus* grown on glucose, methanol, kerosene or sodium formate as the sole carbon source. Bemmann et al. (1978) studied the cultivation of *A. niger* in n-alkanes.

Fungi recovered from solar-treated soil

The number of genera and species obtained from solar-treated soil (8 genera, 19 species and 1 variety) was markedly higher than that obtained from benzene or kerosene-treated soils on glucose-agar. (Tab. 1). The total fungal count was regularly depressed than the control one at both doses after all the experimental periods. This is probably due to the toxic effect of solar. *Aspergillus* (6 species and 1 variety), *Emmericella* (1 species), *Fusarium* (3 species), *Mucor* (2 species), *Myrothecium* (1 species), *Penicillium* (4 species), *Rhizopus* (1 species) and *Trichoderma* (1 species) were recovered from solar-treated soils at both doses (Tab. 1). On solar-Czapek's agar, *Amorphotheca resiniae* was isolated at 40% after 30 and 60 days from solar-treated soil (Tab. 2). Hettige & Sheridan (1984) reported that, 12 monthly samples of diesel fuel from two bulk storage tanks were examined for the presence of fungal contamination during 1982-1983. They isolated over 25 fungal species of which *Cladosporium resiniae* was the predominant fungus occurring in 98% of the samples followed by *Penicillium* spp. (93%). Davies & Westlake (1979) reported that oil-utilizing fungi were isolated from both oil-polluted and uncontaminated northern canadian soils using stationary enrichment technique. Twenty-eight out of the 34 fungi of types isolated were capable of growing on a variety of crude oils. The most frequently isolated species produced abundant small conidia, e.g. *Penicillium* and *Verticillium* spp. and are typical of genera which would normally be expected to grow on soil dilution plates. They also stated that 40 fungal types or strains have been shown to grow on whole crude oils. These included *Torulopsis* sp., *Beauveria bassiana*, *Chryso sporium* sp., *Paecilomyces* sp., *Penicillium* spp., *Trichoderma viride*, *Verticillium* spp., *Acremonium* sp., *Aspergillus niger*, *A. ochraceus*, *A. versicolor* and *Cladosporium* spp. However, the role that fungi play in the decomposition of oil in soil is unknown (Davies & Westlake, 1979). Mycelial organisms can penetrate insoluble substances such as oil and this increases the surface area available for bacterial attack. On the other hand, the studies of Walker & Colwell (1974 a, b) and Walker et al. (1975) showed that although bacteria initiated the degradation of a synthetic petroleum mixture, twice as much was degraded when bacteria, fungi and yeast were present. This synergism between microorganisms could result in an increase in the rate and amount of oil degraded than would be achieved individually. In Egypt, most of the preceding genera and species were isolated previously from oil polluted soils (Hemida, 1991; Bagy, 1992) and from naphthalene-anthracene utilizing microorganisms (Bagy et al., 1992).

In conclusion, the preceding results revealed that, numerous fungal species are able to utilize benzene or kerosene or solar. There are no hydrocarbon utilizing fungi characteristics of benzene, kerosene and solar.

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